

2



TEL AVIV UNIVERSITY אוניברסיטת תל-אביב

SACKLER FACULTY OF MEDICINE  
CHAIM SHEBA MEDICAL CENTER, ISRAEL  
MAURICE AND GABRIELA GOLDSCHLEGER  
EYE RESEARCH INSTITUTE

הפקולטה לרפואה ע"ש סאקלר  
המרכז הרפואי ע"ש שיבא  
המכון לחקר העין  
ע"ש מוריס וגבריאלה גולדשלגר

TEL (03) 53229 50

July 15, 1991

AD-A239 046



Chief.  
Division of Ocular Hazards  
Letterman Eye Institute of Research  
Presidio of San Francisco  
CA 94129-68700  
U.S.A.

Re: Contract No. DAWD 17-87-G-7039  
Final report - September 30 1987 - September 30 1990.

Dear Bruce,

Enclosed please find a final report covering the period September 30 1987 through September 30, 1991 dealing with laser induced retinal injury.

I hope you find this final report satisfactory. Thanks for the cooperation guaranteed by you.

Sincerely yours,

Prof. Nava Haveh, M.D.  
Director  
Maurice and Gabriela Goldschleger Eye Research Institute

NN/mss  
Enc:

DTIC  
ELECTE  
JUL 26 1991  
S D

DISTRIBUTION STATEMENT A

Approved for public release  
Distribution Unlimited

SACKLER FACULTY OF MEDICINE  
25th ANNIVERSARY CELEBRATIONS



חגיגות ה-25  
לפקולטה לרפואה ע"ש סאקלר

המכון לחקר העין ע"ש מוריס וגבריאלה גולדשלגר, המרכז הרפואי ע"ש שיבא, תל השומר 52621, פקסימיליה: 03-5351577  
Maurice and Gabriela Goldschleger Eye Research Institute, Shoba Medical Center, Tel Hashomer 52621, Israel. Fax. 972-3-5351577

41 91-06144



91 7 25 041

**Laser Induced Retinal Injuries**  
**Final Report**  
**(September 30, 1987 through September 30, 1990)**

**Prof. H. Haveh, M.D.**  
**Prof. Michael Belkin, M.D.**

**July, 1991**

**Supported by**  
**U.S. Medical and Development Command**  
**Fort Detrick, Frederick, Maryland 21701-5012**  
**Grant No. DAMD17-87-G-7039**

**Tel Aviv University**  
**Ramat Aviv, Tel Aviv Israel 69978**

Accession For	
NTIS CRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and/or Special
A-1	



Forward

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals": prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission of Life and Sciences, National Research Council (NIH Publication, No. 86-23, revised 1985).

Table of Contents

	<u>Page</u>
Forward .....	2
Summary .....	7
Introduction .....	8
Materials and Methods .....	11
Results .....	15
1) A model for chorio-retinal Leukotriene B <sub>4</sub> production - Pilot study .....	15
2) Choosing the suitable effective antileukotriene drug .....	16
3) Changes in LTB <sub>4</sub> in the vitreous and chorioretina of laser injured eyes .....	17
4) Effect of NDGA an antileukotriene drug on leukotriene B <sub>4</sub> vitreal levels in laser injured eyes .....	17
5) Repeated laser exposure - changes of the inflammatory response ...	18
6) The histopathological changes of laser injured eyes, treated with NDGA .....	19
7) The histopathological changes of laser injured eyes treated concomittantly with NDGA and steroid .....	21
Conclusive remarks .....	22
Clinical military significance .....	
Tables .....	25
Figures .....	25
Bibliography .....	34

CLASSIFICATION OF THIS PAGE

-4-

# REPORT DOCUMENTATION PAGE

1a. SECURITY CLASSIFICATION Unclassified			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release		
4. CLASSIFICATION/DOWNGRADING SCHEDULE			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION Tel-Aviv University			6b. OFFICE SYMBOL (if applicable)		7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Ramat-Aviv Tel-Aviv, ISRAEL 69978			7b. ADDRESS (City, State, and ZIP Code)		
8a. NAME OF FUNDING/SPONSORING ORGANIZATION USAFHRC		8b. OFFICE SYMBOL (if applicable)		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick, Frederick MD 21701-5012			10. SOURCE OF FUNDING NUMBERS		
			PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.
			WORK UNIT ACCESSION NO.		
11. TITLE (Include Security Classification) LASER INDUCED RETINAL INJURIES					
12. PERSONAL AUTHOR(S)					
13a. TYPE OF REPORT		13b. TIME COVERED FROM TO Sept.		14. DATE OF REPORT (Year, Month, Day)	
				15. PAGE COUNT 28	
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	Vitreous protein levels, Neodymium:YAG laser, Leukotrienes B <sub>4</sub> .		
19. ABSTRACT (Continue on reverse if necessary and identify by block number)					
<p>In our previous study we have shown that the ocular response to laser induced ocular injury is characterized by an inflammatory reaction with enhancement in prostaglandin (PG) production and a cellular infiltration. Steroid treatment curtailed the PGs response but did not effect the inflammatory infiltrate. In the present study, we investigated the involvement of leukotrienes B<sub>4</sub> (LTB<sub>4</sub>) in laser induced retinal injury. The following parameters were studied; LTB<sub>4</sub> production by the retina-</p>					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL Rebecca McHenry			22b. TELEPHONE (Include Area Code) 415/561-4893		22c. OFFICE SYMBOL

DD FORM 1473, 84 MAR

83 APR edition may be used until exhausted.  
All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

SECURITY CLASSIFICATION OF THIS PAGE

choroid, its accumulation in the vitreous, and changes in vitreal protein levels. In addition, the effect of an antileukotriene drug (NDGA) on LTB<sub>4</sub> accumulation in the vitreous was studied.

Histopathological evaluation of the inflammatory response following NDGA treatment alone or combined with steroids was performed well.

The antileukotriene drug that has been chosen for the present study was norguaiaretic acid (NDGA), because it was more efficient than BW755C (another antileukotriene drug studied).

Initially, the amounts of LTB<sub>4</sub> produced by the retinal and released into the vitreous in vitro were too small to be detected by the biochemical assay used by us. To overcome this problem, a model for the study of LTB<sub>4</sub> production by the retina-choroid was established, using Ca<sup>+2</sup> ionophore A13187 to augment production so that we achieved levels measurable by our biochemical techniques. Our results indicate that LTB<sub>4</sub> production and its vitreal accumulation, as well as vitreal protein levels were elevated in eyes exposed to Nd:YAG laser irradiation during a two week period. It is our suggestion that augmented LT levels following laser induced trauma might be responsible for the immediate incapacitation and the late vision reduction.

Measures to minimize leukotrienes production by drugs so as to attenuate the visual incapacitation included the use of two therapeutic regimens: NDGA given alone or concomitantly with steroids. Treatment involving NDGA alone (applied intramuscularly daily) effectively reduced LTB<sub>4</sub> accumulation in the vitreous but it had no effect on the inflammatory cellular infiltrate at and around the laser lesion site. Similarly, the chorio-retinal scarring and the retinal pigment epithelium were unaffected by this treatment. The combined treatment of NDGA and steroid resulted in absence of inflammatory cellular infiltrate which are numerous in untreated eyes. Additionally, the chorio-retinal damages at the laser lesion site was minimal and affecting outer nuclear layer alone with preservance of the layers above. It is noteworthy that the retinal pigment epithelium at the surrounding area was less damaged in these eyes. We can conclude that

SECURITY CLASSIFICATION OF THIS PAGE

SECURITY CLASSIFICATION OF THIS PAGE

treatment of laser exposed eyes with NDGA alone effectively prevented the excessive levels of vitreal  $LTB_4$ , but left the cellular inflammatory infiltrate unchanged. However, the combined treatment of NDGA and steroids effectively lessened the chorioretinal damage and lack of cellular inflammatory reaction, resulting in minimal retinal damage.

SECURITY CLASSIFICATION OF THIS PAGE

Summary

This final report covers the work performed from September 30, 1987 through September 30, 1990, and includes additional data obtained during a period of non-funded extension during February 1987 through September 1987.

During the period covered by this final report we:

- 1) Established a reproducible model for the study of leukotriene B<sub>4</sub> production by retina-choroid, in laser injured eyes.
- 2) Compared the efficacy of two antileukotriene drugs (NDGA and BW755) and chose NDGA as the drug of choice for the treatment of laser injuries.
- 3) Studied changes in leukotriene B<sub>4</sub> levels in the vitreous of laser injured eyes.
- 4) Studied the effect of NDGA on laser injured eyes as manifested by changes in vitreal leukotriene B<sub>4</sub> response.
- 5) Studied the effect of repeated laser exposure on the inflammatory response.

Histopathological changes of laser injured eyes following two therapeutic regimens;

- 6) NDGA administration daily (intramuscular 10mg/Kg body weight),
- 7) A combined treatment of steroids and NDGA; Daily intramuscular injections of NDGA (10 mg/kg body weight) and dexamethasone (0.1 mg/kg body weight).



### Introduction

Our previous study of "Steroid treatment of a laser induced retinal injury" terminated in 1987, demonstrated that Neodymium (Nd):YAG laser retinal injury was associated with an inflammatory reaction at the laser lesion site; Laser induced inflammation was manifested by an extensive polymorphonuclear (PMN) infiltration and an excessive production of prostaglandin (PG) type  $E_2$  by the retina-choroid. Our findings of an increase in  $PGE_2$  vitreal levels in lasered eyes to above pre-laser values, and an enhanced vitreal protein concentration were indicative of a "break" in the blood retinal barrier, a condition closely related with ocular inflammatory response. Subsequently, after retinal laser exposure, the injury site underwent scarring, which involved chorioretinal adhesion, and loss of photoreceptors leading to impaired retinal function.

In our previous study, steroid treatment which was started immediately after laser exposure was only partially effective in reducing the chorioretinal response to trauma, but it significantly curtailed the vitreal reaction; as it inhibited altogether the enhancement in vitreal  $PGE_2$  levels. Additionally, steroid treatment of laser injured eyes had a considerable transitory inhibitory effect on protein leakage into the vitreous. This treatment had no apparent effect on the inflammatory cellular infiltration.

The inefficacy of steroid treatment to abolish completely the laser induced chorio-retinal inflammatory response established the need for a search for a better and more effective therapeutic regimen. An effective therapeutic regimen was aimed at minimizing the ocular inflammatory response so that the immediate incapacitation as well as the subsequent scarring process which later impairs vision will be diminished.

The requirement for a new measure to reduce the laser induced inflammatory response led us to look into the behavior of leukotrienes  $B_4$  ( $LTB_4$ ) known as inflammatory mediators of the ocular response to trauma (1).

Our hypothesis that laser induced retinal injury might be associated with excessive leukotriene (LT) production, was based on the fact that enhanced PGs production is related to an increased availability of free arachidonic acid; which serves also as a substrate for LT production (1).

Increased levels of LT type ( $LTB_4$ ) in the aqueous humor of rabbits was demonstrated following ocular trauma (2-4), in uveitis in humans (5), a known ocular inflammatory condition. The significance of LTs in mediating inflammatory reaction in various organs is exemplified in studies in which LT antagonists or antileukotriene drugs, improved the clinical outcome in situations such as endotoxemic shock or various forms of trauma (6,7). LTs generated in the brain affect cerebral circulation to promote vasospasm and edema (8,9). The retina, which is also a nervous tissue might be affected in a similar manner, so that its exposure to excessive LT levels might have a detrimental effect on vision.

In order to assess the mediatory role of LT in laser induced retinal injury, we studied in these eyes; (1) changes of  $LTB_4$  levels in the vitreous body, as well as its production by the chorioretina, (2) studied the effect of two antileukotriene drugs, Norguaiaretic acid (NDGA) and BW755C, on vitreal and chorioretinal  $LTB_4$  levels in laser injured eyes. NDGA inhibits LT production through inhibition of the lipoxygenase pathway and has no effect on PGs formation (10-12). BW755C affects simultaneously both the formation of LT and PGs, (3) studied histopathological changes following daily treatment with NDGA (10 mg/Kg body weight) alone, or when administered concomitantly with steroids (dexamethasone 0.1 mg/Kg body weight).

However, in any pathophysiological process in which therapeutic LTs and PGs have been argued to participate, many other biologically active substances including thromboxanes, oxygen radicals, interleukines, etc. are also produced in excess. Therefore, it is expected that successful therapeutic strategies would include drugs aimed against multiple mediators, in addition to modulation of the LT and PGs actions (11,12).

### Materials and Methods

Pigmented rabbits (a total of 243 rabbits) of either sex, weighing 2-2.5 Kg were used. They were anesthetized by 35 mg/Kg Ketamine (Ketalar) and 5 mg/Kg Xylazine (Rompun) injected intramuscularly (i.m.).

### Laser procedure

Neodymium:YAG laser exposure - A Q-switched Neodymium (Nd):YAG laser (Lasag:Thun, Switzerland) was used to perform 15 retinal laser applications through a Russel-Fankhouser three mirror lens. These applications were aimed at the posterior pole of the right eye of each rabbit below the optic nerve head, at areas devoid of blood vessels, as far apart as possible. The other eye was left untreated. The endpoint in establishing an Nd:YAG laser burn a visible whitening of the retina at the burn site with slight reddening of the underlying choroid. Excluded were eyes with retinal or vitreal hemorrhage. Each exposure consisted of a single pulse of single burst in the multimode at energy level of 0.1-0.4 millijoules.

In vivo studies: Thirty minutes before the laser procedure, the animals were anesthetized by 35 mg/kg Ketamine and 5 mg/kg Xylazine injected (i.m.) following pupil dilatation with Tropicamide 0.5%. Local anaesthesia with Benoxinate 0.1% preceded the laser treatment. Following animals sacrifice, with intravenously injected overdose of pentobarbiturate, the right eye of each rabbit was enucleated, and samples of the vitreous body and the retina-choroid were obtained for  $LTB_4$  and protein measurements as described.

Retina-choroid preparation Following enucleation, the corneas were cut all around the limbus, and the iris and ciliary body were removed by pulling gently at the iris base. The lens was removed and the vitreous expelled as described, and placed separately in a vial. The retina attached to the

choroid (a retina-choroid preparation) was separated from the sclera, and each preparation was placed in a vial containing the buffer for further studies on LT production, described below.

In vitro studies - Each retina-choroid preparation was incubated separately for 30 minutes at 37°C in a shaking bath in 0.6 ml of a Krebs Hepes Buffer, Ph 7.4, with or without the addition of  $\text{Ca}^{+2}$  ionophore A23187 at various concentrations (0.1 to 5.0 micromoles). At the end of the incubation period a sample was withdrawn for  $\text{LTB}_4$  determination.

The antileukotriene effect of NDGA and BW755C on chorioretinal  $\text{LTB}_4$  production was studied using a retina-choroid model previously described, so that in this set of experiments we used a retina-choroid preparation incubated in  $\text{Ca}^{+2}$  ionophore containing media. The appropriate drug was also added to the incubation medium using various doses (see results section). At the end of a 30 minute incubation period, samples were withdrawn for  $\text{LTB}_4$  determination. The amounts which accumulated in the incubation medium containing the retina-choroid was considered as representing the amounts produced by the tissue, and this was dubbed - " $\text{LTB}_4$  in vitro production".

Leukotriene B<sub>4</sub> determination - The retina-choroid preparation was incubated in 0.6 ml Krebs Ringer Bicarbonate Hepes Buffer, pH 7.4 in a period, the tissue was removed and samples were withdrawn for  $\text{LTB}_4$  determination. The vitreous of each eye was similarly incubated following the addition of 0.3 ml of the same buffer. At the end of the incubation period, a sample was withdrawn for  $\text{LTB}_4$  determination.  $\text{LTB}_4$  was determined using a Radioimmunoassay kit (New England Nuclear) with a specific antibody.

Protein determination - Protein was measured in the vitreous body using the modified Lowry method (13).

#### Experimental design

A total of 251 pigmented rabbits of either sex (2 to 2.5 kg) were used. Rabbits were divided into seven study groups (48,60,41,30,40,16 and 16 rabbits in each group, respectively). Each laser irradiated group was further divided into 4 subgroups according to follow up after laser exposure (1, 3, 7 and 14 days after laser exposure). An additional unexposed group served as control.

Unexposed control groups - Control rabbits were not exposed to any laser irradiation and their vitreous and retina-choroid of the right eye of each rabbit were obtained for  $LTB_4$  determination as described for the Nd:YAG laser group.

Nd:YAG laser exposed groups - Rabbits which underwent Nd:YAG laser irradiation were divided into 4 subgroups, studied at 1, 3, 7 and 14 days after exposure.

The following were the 7 main subjects studied;

- (1) Study on retinal in vitro  $LTB_4$  production.
- (2) Comparison of the effect of two antileukotriene drugs on  $LTB_4$  in vitro chorioretinal production.
- (3) Study on changes in retinal and vitreal  $LTB_4$  levels of laser exposed eyes.
- (4) Study on the effect of NDGA on vitreal  $LTB_4$  of laser exposed eyes.
- (5) Study on repeated laser exposure.
- (6) Histopathological study on the effect of NDGA (10mg/kg body weight, i.m.) in laser injured eyes.
- (7) Histopathological study on the effect of the combined treatment with NDGA and steroids [NDGA (10mg/kg body weight) i.m. and dexamethasone (0.1mg/kg body weight), respectively].

Study groups:

In the first two study groups - In vitro study of leukotrienes by retina-choroid (group 1), and Comparison of the in vitro inhibitory effect of two antileukotriene drugs (group 2), the in vitro changes in  $LTB_4$  production by retina-choroid was studied.

Changes of the chorio retinal and vitreal  $LTB_4$  response of laser injured eyes (group 3) were studied in vivo, in two subgroups of rabbits; an unexposed control group and a laser exposed group. In the laser exposed group, the retina-choroid was obtained at 1, 3, 7, and 14 days after exposure and then incubated, as described. Samples from the incubation media containing the retina-choroid were withdrawn for determination of  $LTB_4$ , which represented the in vitro production. Samples from the vitreous were used for determination of vitreal protein and  $LTB_4$  levels.

The in vivo effect of an antileukotriene drug (NDGA) on the vitreal  $LTB_4$  response in laser exposed eyes (group 4), over a two week period, was studied in laser exposed eyes, which has been treated with NDGA, which was administered i.m. within one hour post laser. This treatment was repeated daily.

Effect of repeated laser exposure on  $LTB_4$  vitreal response (Group 5), was studied in animals exposed to laser which were exposed to another session of laser one week later, and this time the laser applications were aimed at areas free of visible laser lesions. The animals were sacrificed at 1, 3, 7 and 14 days after the second laser exposure.

Histopathological changes in laser injured eyes following NDGA treatment (Group 6) - Laser procedure followed that used in the other groups, and the rabbits were treated daily with NDGA (10mg/Kg body weight i.m.) over a two week period. The eyes were fixed in 10% formaldehyde.

Histopathological changes in laser injured eyes following the combined treatment of steroids and NDGA (Group 7) - laser procedure followed our routine and the treatment consisting of dexamethasone (0.1mg/Kg body weight) and NDGA (10 mg/Kg body weight) was started within 1 hour post laser exposure. Infections were repeated daily.

### Results

#### 1) Study of the in vitro chorio-retina LTB<sub>4</sub> production. (Group 1)

LTB<sub>4</sub> in vitro production by the chorio-retina yielded amounts too small to be detected by our radioimmunoassay. In order to enhance retinal LTB<sub>4</sub> in vitro production; Ca<sup>+</sup> ionophore A13187 was added to the incubation medium; this drug is widely used as an activator of LTB<sub>4</sub> production. The effect of varied concentrations of Ca<sup>++</sup> ionophore (from 0.1 to 5.0 micromoles) was studied in 48 rabbits divided into 6 groups (Table 1).

The addition of Ca<sup>+</sup> ionophore (0.1 to 1.0 micromoles) caused a dose dependant increase in retinal LTB<sub>4</sub> production (Table 1), and maximal in vitro production levels were achieved at 1 micromole. With greater concentrations of Ca<sup>2+</sup> ionophore, LTB<sub>4</sub> production was enhanced to a lesser degree and at 5 micromolar of the ionophore, no excitatory effect was noted. Following the addition of 1 micromole, Ca<sup>2+</sup> ionophore LTB<sub>4</sub> in vitro production levels reached levels of 389±132 pg/gm wet weight (Table 1) which were easily detected by our biochemical assay. We found this method of using Ca<sup>2+</sup> ionophore during in vitro chorioretinal LTB<sub>4</sub> production studies, acceptable for our use.



2) Comparison of the in vitro efficacy of two antileukotriene drugs (BW755C and NDGA). (Group 2)

The study of the inhibitory effect of the two antileukotriene drugs - BW 755C and NDGA on  $\text{LTB}_4$  in vitro production by the chorio-retina helped to decide their relative efficacy: It was our hypothesis that the drug possessing a greater inhibitory effect on retinal  $\text{LTB}_4$  production, is likely to be more active as an antiinflammatory agent in this tissue.

Comparison of the two antileukotriene drugs involved 60 rabbits, using various doses. Each drug was studied at 3 different concentrations (0.1, 1.0 and 10 micrograms/ml). The appropriate drug was added on initiation of incubation period, to a vial containing  $\text{Ca}^{+2}$  ionophore (1.0 micromole) and a chorio-retina. Following a 30 minute incubation period in  $37^\circ\text{C}$  in a shaking bath, a sample from the media was withdrawn for  $\text{LTB}_4$  determination.

In evaluating our results, we considered as baseline the amounts of  $\text{LTB}_4$  produced by chorio-retina treated with  $\text{Ca}^{+2}$  ionophore only, but with no other drug added ( $352 \pm 128$  per/gr. wet wt.). Both BW755C and NDGA had a significant inhibitory effect on  $\text{LTB}_4$  production which was evident at each of the concentrations studied (Table 2). NDGA at low concentration (0.1 microgram/ml) inhibited baseline  $\text{LTB}_4$  production by 90% and was more effective than the corresponding BW755C concentration ( $27 \pm 13$  and  $107 \pm 30$  pg/gm wet wt., respectively). At higher dose (1.0 microgram/ml) both drugs exhibited a similar effect, demonstrating an inhibitory effect which reduced levels to less than 10% baseline values. The standard deviation in the two latter groups were equal or greater than the mean and this was caused by the fact that production ranged from 0 to 40. Based on our data, we decided to use NDGA as our drug of choice for inhibition of  $\text{LTB}_4$  production in our animal studies.

3) Changes in vitreal and chorio-retinal  $LTB_4$  in laser injured eyes.  
(Group 3)

The determination of  $LTB_4$  in the chorio-retina and vitreous of eyes subjected to Nd:YAG laser irradiation encountered two main problems: The the problem of naturally low leveled chorioretinal production was overcome by the addition of  $Ca^{+}$  ionophore A13187 to the media of the incubated retina, following the method described in "Results", section A.

The second problem of low vitreal  $LTB_4$  levels was solved by increasing the number of laser applications to 15, using the same power setting (method section, laser procedure).

Baseline (pre-laser) vitreal content of  $LTB_4$  was very low and ranged from 0 to 33 pg/gm weight (wt) with an average of  $19 \pm 9$  pg/gm wt. Following laser exposure, vitreal  $LTB_4$  levels were elevated to above pre-laser values and peaked on day 3, at which time they were 280% higher than pre-laser values (Figure 1).

Nd:YAG laser induced retinal damage was associated with an enhanced chorioretinal  $LTB_4$  production throughout the first week following exposure. An augmented production (200% higher than baseline) was evident already at the first day (Fig. 1), and remained elevated also on day 3. Maximal production values were achieved on day 7 and reached levels 2.7 times higher than pre-laser values. Control levels were resumed on day 14.

4) Effect of an antileukotriene drug - NDGA on retinal laser injury.  
(Group 4)

The effect of an antileukotriene drug - NDGA on the ocular inflammatory response following retinal laser exposure was studied using the following parameters: the changes in  $LTB_4$  in vitro production by the chorio-retina and its accumulation in the vitreous.

The decision to use NDGA as our antiinflammatory agent, was based on our previous work (see group 2) which exhibited the supremacy of the NDGA.

In assessing our results, the pre-laser values were considered as baseline (100%) and levels in the treated and untreated groups were given as percentage of baseline. Our findings show that NDGA treatment on the vitreal  $LTB_4$  response in laser exposed eyes (Fig. 2) indicate that NDGA reduced the accumulation of  $LTB_4$  in the vitreous during the first week after exposure, but at two weeks a progressive elevation of  $LTB_4$  levels was noted.

5) Repeated laser exposure. (Group 5)

This study on exposure to repeated laser irradiation might be of military significance; Laser exposure at low energy levels, might go unnoticed during the initial phase (especially if the lesion site does not include the macular area). Therefore, the unaware soldier will be sent back to combat where he might be exposed to additional laser irradiation. We suggested that repeated laser exposure even to low energy levels might result in an accumulative damage with a progressive accumulation of toxic substances in the vitreous body and a subsequent reduction in visual acuity.

In the present series of experiments, the animals were lased once and one week later were exposed to a second similar session of laser irradiation. During the second laser exposure, the laser was aimed at areas free of visible laser lesions (as described under experimental design). Following the second laser exposure, the animals were sacrificed, at various time intervals, Leukotriene  $B_4$  was determined in the media containing the chorioretina or the vitreous of each eye, while protein was measured only in the vitreous as described.

Our results (Fig. 3) demonstrate that repeated laser exposure causes an increase in  $LTB_4$  vitreal concentrations to above pre-laser values. Peak

levels, 7 and 4.5 folds higher than baseline were noted during the second week after exposure (days 7 and 14 respectively), while earlier levels did not exceed pre-laser. The pattern of changes of vitreal  $\text{LTB}_4$  content in eyes subjected to repeated exposure was different from that observed in eyes exposed to the laser only once; In the latter group, vitreal  $\text{LTB}_4$  values peaked earlier after exposure and maximal levels were lower. In eyes subjected to repeated exposure baseline levels were resumed during the second week (Fig. 3).

This pilot study serves as a partial confirmation to our suggestion that repeated laser exposure to low energy levels might be associated with a more pronounced inflammatory vitreal response than that observed in eyes exposed to laser only once. This enhanced  $\text{LTB}_4$  response occurring late after exposure, might indicate that repeated laser exposure could be unexpectedly harmful to the eye.

6) Histopathological changes following NDGA treatment of laser injured eyes (Group 6)

One day after laser exposure

At the retinal laser lesion site, the retina was severely damaged and a "hole" was formed (Fig. 4). The inner part of the retina is affected, and show numerous areas of separation from the inner limiting membrane. A moderate pre-retinal hemorrhage is also present, but with no edema or inflammatory cellular infiltrate. Destruction of the photoreceptor layer is evident by the presence of vacuoles (Fig. 5). It is noteworthy that in the anterior segment of the eye (iris, trabeculum, and ciliary body) a pronounced inflammatory cellular infiltration was noted (Fig. 6).

Three days after laser exposure

The choroid beneath the laser burned area is infiltrated by few inflammatory cells. At the laser lesion site the photoreceptors' damage

remained unchanged with detachment of the middle limiting membrane which is usually associated with an irreversible damage to the retina. At the area surrounding the laser burn the retina is affected in a rather peculiar way - it demonstrates folding of the retina over an area 2 to 3 disc diameter around the lesion (Fig. 7). At this area (peripheral to the lesion) the inner limiting membrane remains tight with no folding. However, layers underneath the inner limiting membrane were damaged. Additionally, the optic nerve head shows severe papillitis with numerous inflammatory cells (Fig. 8). This indicates that the detrimental effect of laser exposure is not localised but extends to far beyond the laser application.

At three days following exposure, the area surrounding the burn is extensively affected, showing retinal folding, destruction of the inner limiting membrane layer, but with little evidence of inflammatory cellular infiltration at the choroid by inflammatory cells.

Seven days after exposure

The inflammatory reaction at the anterior segment has completely subsided. The retina at the laser lesion site demonstrates a choroidal and retinal infiltration by inflammatory cells, and a localized area of chorio-retinal adhesion (Fig. 9). The retinal pigment epithelial cells in the surrounding area are sloughing off into the subretinal space. At the areas surrounding the laser lesions, retinal folding still persists but the outer nuclear layer is not affected and that area might resume normal function in 3 to 6 months. No inflammatory cellular infiltration is noticeable at the lesion periphery.

Fourteen days after exposure

The laser lesion site is still elevated due to subretinal fluid accumulation, and the retinal cellular elements can not be discerned. No chorioretinal adhesion is evident at the surrounding area, but vacuolization

of the photoreceptor layer is evident (Fig. 10) associated with sloughing off of the RPE layer and inflammatory choroidal infiltration (Fig. 11).

An infiltrate under the inner nuclear layer at the area surrounding the laser lesion site is indicative of damage to the Muller cells (Fig. 12). The fact that the photoreceptor are still adherent to the RPE might serve as an indication that these changes might be reversible. Thus, improvement in vision capacity in these regions is plausible.

7) The effect of concomittant therapy of NDGA and steroids -  
Histopathological study (Group 7)

Histopathology, the combined treatment of steroid and NDGA affected the appearance of the chorioretinal scarring at the laser lesion site, and the associated inflammatory response. At the chorioretinal adhesion the outer nuclear layer is destroyed (Fig. 13) however the layers above it preserve their characteristic appearance. Additionally, the area surrounding the laser burn is very slightly affected by the damage. The most striking finding is the absence of inflammatory cellular infiltrate in the underlying choroid or at the laser lesion site and the surrounding areas. The minimal scarring in these treated eyes can not explain that the lesion was smaller due to lower energy laser irradiation. For vitreal hemorrhage is visible in the adjacent vitreous (Fig. 14) indicating bleeding of ruptured choroidal blood vessels, due to strong laser application effective enough to affect the retina as well as the choroid.

At higher magnification the lack of any inflammatory cellular infiltrate is obvious, while the retinal pigment epithelium is still adherent to the Bruch's membrane and is not sloughed off. The overlying outer nuclear and plexiform layers are severely affected, however, no scar formation is evident (Fig. 15). An adherence between the retinal pigment epithelium and the external limiting membrane is noticeable.

In summary, in laser exposed eyes, treated concomitantly by NDGA and steroids no inflammatory cellular response was apparent at the laser lesion site. The burn revealed only damaged outer nuclear layers with minimal scar formation. The chorioretinal scarring in eyes treated by combined steroids and NDGA was significantly less than in NDGA treated eyes, with practically no inflammatory cellular response during a one week follow up.

#### Conclusions

Findings obtained during the present study might be of interest, as they throw light on the ocular response to nonchemical traumatic stimuli and therapeutic measures to attenuate the chorioretinal damage. These findings have a special bearing because of their military significance as they point to which are the mediators determining the course of laser induced inflammation, and most importantly - laser induced retinal scarring.

Our data might also be helpful in contemplating a new therapeutic approach to retinal laser injury.

The conclusions derived from our findings involve the following:

(1) Leukotriene B<sub>4</sub> involvement in retinal laser injury: Laser induced retinal injury is associated with a significant elevation in LTB<sub>4</sub> production by the injured chorio-retina. Chorioretinal LTB<sub>4</sub> formation of lasered eyes exceeded pre-laser values throughout the first week following exposure, but resumed baseline by day 14. A possible source for chorioretinal LTB<sub>4</sub> formation are the severely damaged retinal layers at the laser lesion site, and at the surrounding areas. It is well established that nervous system of which the retina is an integral part reacts by activation of the lipoxygenase pathway leading to excessive LT production. Another possible source for LTB<sub>4</sub> might be the inflammatory cellular infiltrate, known for their ability to produce LTB<sub>4</sub>.

Additionally, we demonstrated in laser injured eyes a two-fold increase in  $\text{LTB}_4$  vitreal concentration, which persisted throughout the first week after exposure. In the intact eyes vitreal  $\text{LTB}_4$  levels are kept at very low concentration by an active transport mechanism located at the blood retinal barrier.  $\text{LTB}_4$  produced in excess by the lasered chorio-retina are released into the vitreous. Their accumulation in the vitreous to above pre-laser value is the result of excessive production and an impaired removal mechanism out of the vitreous (impaired blood retinal barrier activity). Another aspect of  $\text{LTB}_4$  vitreal accumulation is the fact that they act as noxious stimuli on the retina causing vasodilatation, increased permeability of blood vessels with subsequent exudation etc., starting up a vicious cycle. Therefore, therapeutic measures should be aimed at minimizing the storage of  $\text{LTB}_4$  in the vitreous.

(2) NDGA effect on laser induced inflammatory response; NDGA treatment of laser exposed eyes resulted in a significant decrease in vitreal  $\text{LTB}_4$  levels. However, NDGA treatment had no effect on the inflammatory cellular response at the laser lesion site. It did not change also significantly the chorio-retinal scarring at the laser site, and the extensive retinal pigment epithelial damage at the adjacent areas.

(3) Combined steroids and NDGA treatments effect on the histopathological inflammatory response. Treatment of laser exposed rabbits concomitantly with NDGA and steroids resulted in complete absence of an inflammatory cellular response. This was not achieved by the separate use of either steroids or NDGA. In addition the combined treatment resulted in less scarring, following treatment of the chorio-retinal adhesion an adherence of the retinal pigment epithelium to the external limiting membrane was



noted with preservice of the inner layers and the surrounding pigment epithelium layer. Therefore scarring was minimal and localized, with less damage to adjacent areas.

**TABLE 1:**

The effect of  $\text{Ca}^{++}$  ionophore A3187 on the in vitro production of Leukotriene  $\text{B}_4$  by chorio retina of intact eye

$\text{Ca}^{++}$ ionophore concentration (micromole)	0.1	0.3	0.5	1	2	5
Retinal Leukotrienes levels (pg/gr wet wt) (mean $\pm$ SD)	123 $\pm$ 50	316 $\pm$ 60	285 $\pm$ 134	389 $\pm$ 132	93 $\pm$ 76	0
*Control Leukotriene levels (pg/gr wet wt)	12 $\pm$ 7	12 $\pm$ 3	10 $\pm$ 4	9 $\pm$ 4	15 $\pm$	12 $\pm$ 3

\* Retinal leukotrienes production by retina-choroid untreated by  $\text{Ca}^{++}$  ionophore. Each group included 8 animals..

**TABLE 2:**

A comparison of the inhibitory effect of two antileukotriene drugs (BW 755C and NDGA) on leukotriene  $\text{B}_4$  production by the retina choroid

<u>Concentration</u> (microgram/ml)	<u>*Leukotriene <math>\text{B}_4</math> production by the retina-choroid (pg/gr wet wt) Mean <math>\pm</math> SD</u>			
	<u>BW755C</u>		<u>NDGA</u>	
0.1	107 $\pm$ 30	n=9	27 $\pm$ 13	n=9
1.0	35 $\pm$ 64	n=9	23 $\pm$ 20	n=9
10	0	n=9	0	n=9
Control	352 $\pm$ 128	n=6		

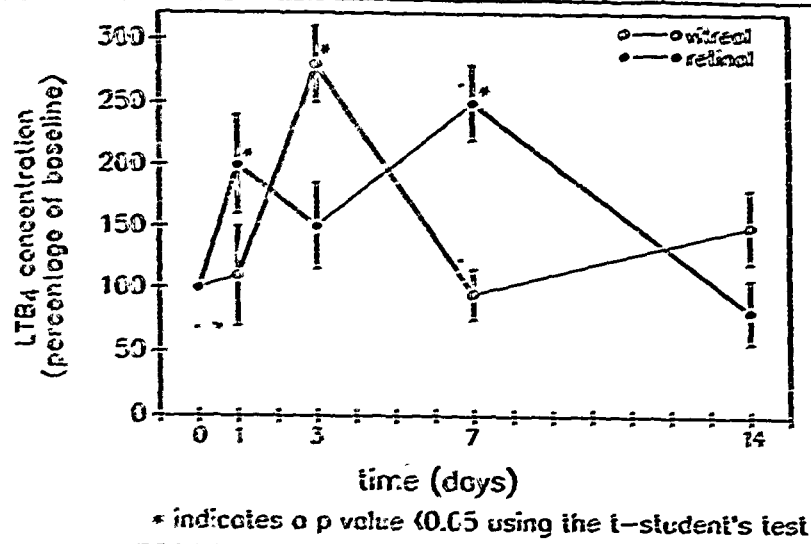


Fig. 1: Leukotriene B<sub>4</sub> in the Vitreous and its in vitro production by the Retina-Choroid

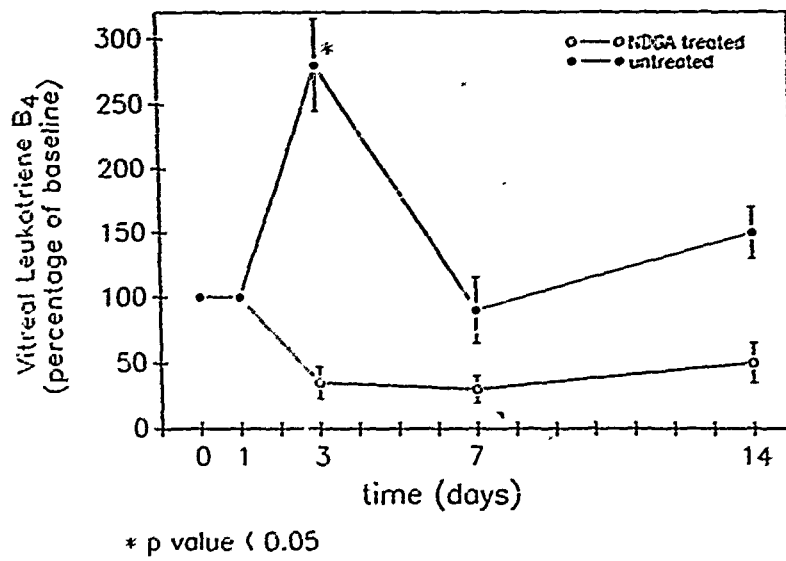


Fig. 2: Vitreal Leukotriene B<sub>4</sub> concentration following treatment by an antileukotriene drug - NDGA

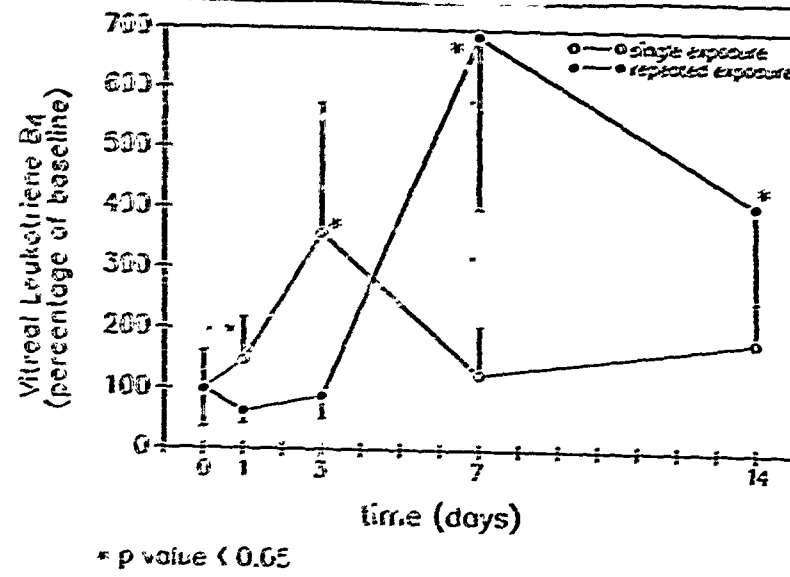


Fig. 3: Vitreal Leukotriene B<sub>4</sub> concentrations following repeated Laser exposure



Fig. 4: A retinal laser lesion at one day post exposure with "hole" formation including the inner nuclear layer



Fig. 5: A retinal laser lesion at one day post exposure, with vacuoles formation at the photoreceptor layer. No inflammatory cellular infiltrate is yet evident



Fig. 6: An inflammatory cellular infiltration, edema at the iris base, trabeculin and ciliary body at 1 day post exposure



Fig. 7: A retinal laser lesion at 3 days post laser showing "folding" of the internal limiting membrane at the surrounding areas

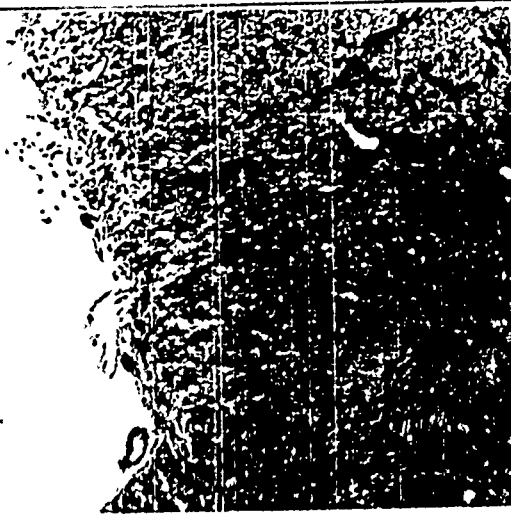


Fig. 8: The optic nerve head at 3 days post retinal induced laser injury, showing inflammatory cellular infiltrate at the superficial and deep layers



Fig. 9: A retinal laser lesion at 7 days post exposure, showing localized chorio retinal adhesion, with sloughing off of the retinal pigment epithelium cells into the subretinal space at the surrounding area

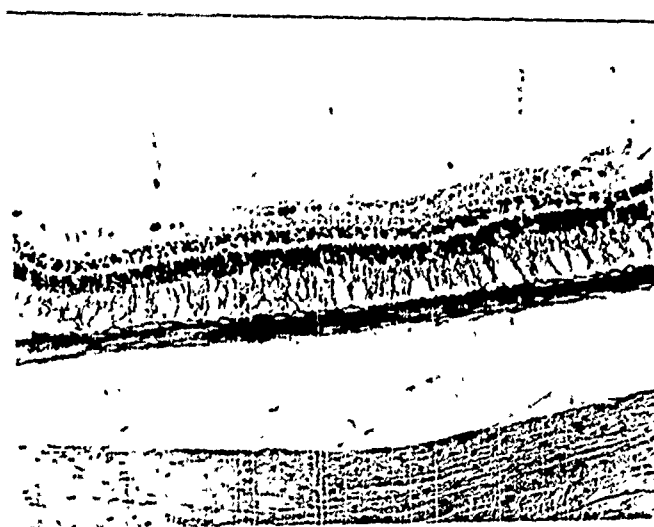


Fig. 10: The area surrounding retinal laser lesion at 14 days post exposure, showing vacuolization of the photoreceptors with sloughed off retinal pigment epithelium and inflammatory cellular infiltrate

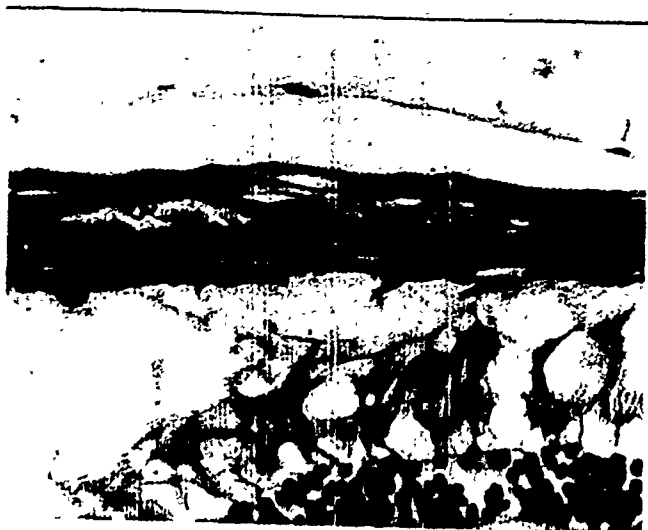


Fig. 11: Sloughing off of the retinal pigment epithelium at an area surrounding the laser lesion site on day 14 post exposure

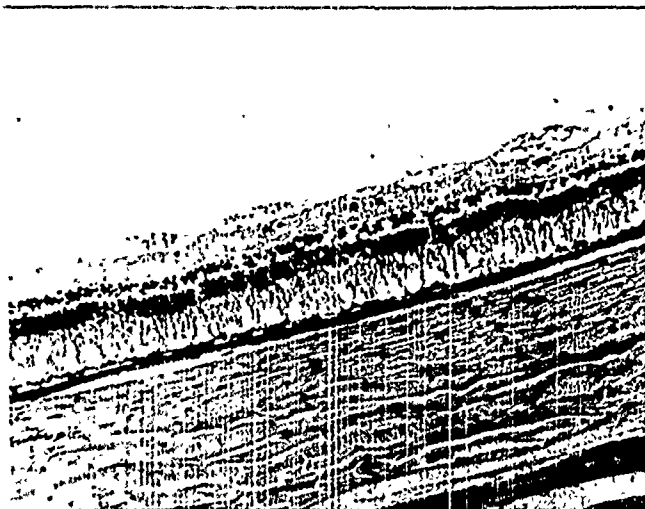


Fig. 12: The area surrounding the laser lesion site on day 14 post exposure showing cellular inflammatory infiltrate indicative of damage to the Muller cells



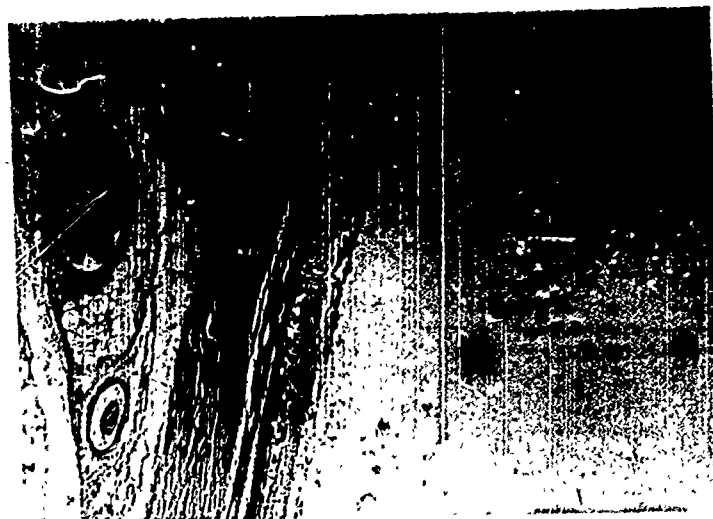


Fig. 13: A retinal laser lesion on day 3 following exposure in eyes treated with combined NDGA and steroids. At the lesion the chorio retinal damage is confined to the outer nuclear layer, while the layers above are preserved. No inflammatory cellular infiltrate are noticeable.

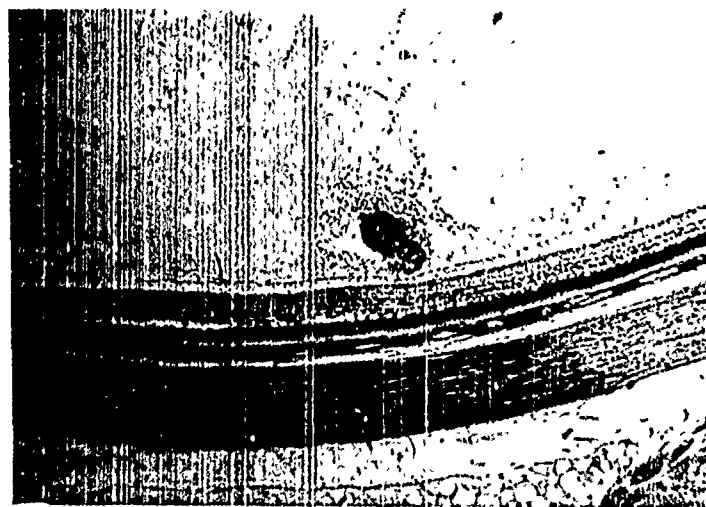


Fig. 14: A vitreal hemorrhage in a lagged eye treated concomitantly with NDGA and steroids - 3 days after exposure.



Fig. 15: Retinal laser lesion site at 3 days post exposure. In eye treated concomitantly with NDGA and steroids. The adherence of retinal pigment epithelium to the middle limiting membrane is noticeable, with minimal "fibrotic" reaction.

Bibliography

1. Samuelsson, B.  
Leukotrienes: A new group of biologically active compounds including SRS-A.  
Trends in Pharmacology Science, 9:227, 1980.
2. Latanza, L., Alfaro, D.V., Bockman, R., Iwamoto, T., Heinemann, M.H., Chang, S.  
Leukotrienes levels in the aqueous humor following experimental ocular trauma.  
Retina, 8:199-204, 1988.
3. Leibowitz, H.M., Ryan, W.Y., Kupperman, A.  
The role of leukotrienes B<sub>4</sub> in PMN chemotaxis of the inflamed cornea.  
Invest. Oph. Vis. Sci. ARVO abstract Suppl., 26:320, 1985.
4. Parker, Y.A., Guetzl, E.Y., Friedlaender, M.A.  
Leukotrienes in the aqueous humor of patients with uveitis.  
Arch. Oph., 104:722-724, 1986.
5. Stjeruscharz, Y., Sherk, T., Sears, M.  
Ocular responses to leukotrienes C<sub>4</sub> and D<sub>4</sub>  
Prostaglandins 27:5-17, 1984.
6. Leffer, A.M.  
Leukotrienes as mediators of ischemic and shock.  
Biochem. Pharmacol. 35:123-127, 1986.
7. Feuerstein, G., Hallenbeck, J.M.  
Leukotrienes in health and disease.  
FA SEB J., 1:186-192, 1987.
8. Black, K.L.  
Leukotriene C<sub>4</sub> induced vasogenic cerebral edema in rats.  
Prostaglandin Leukotriene Med., 14:339-340, 1984.

9. Mayhan, W.G., Sahagun, G., Spector, R., Heistad, D.D.  
Effect of Leukotriene C<sub>4</sub> on the cerebral microcirculative.  
Am. J. Physio., 251:H471-H474, 1986.
10. Murphy, R.C., Hammerstroms, S., Samuelsson, B.  
Leukotriene C: A slow reacting substance from murine mastocytoma.  
Proc. Natl. Acad. Sci. U.S.A., 76:4275-4279, 1979.
11. Piper, P.J., Samhoun, M.N.  
Leukotrienes  
Br. Med. Bull. 43:297-311, 1987.
12. Denzlinger, C., Rapp, S., Hagmann, W., Keppler, D.  
Leukotriene as mediators in tissue trauma.  
Science, 30:330-332, 1985.

DISTRIBUTION LIST

4 copies	Commander Letterman Army Institute of Research (LAIR), Bldg. 1110 ATTN: SGRD-ULZ-RC Presidio of San Francisco, CA 94129-6815
1 copy	Commander US Army Medical Research and Development Command ATTN: SGRD-RMI-S Fort Detrick, Frederick, Maryland 21701-5012
12 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDAC Cameron Station Alexandria, VA 22304-6145
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20814-4799
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM For Sam Houston, TX 78234-6100

---

**SUPPLEMENTARY**

**INFORMATION**

# ERRATA

AD-A-239046

GRANT NO: DAMD17-87-G-7039

TITLE: TREATMENT OF LASER INDUCED RETINAL LESIONS

SUBTITLE: Laser Induced Retinal Injuries

PRINCIPAL INVESTIGATORS: Nava Naveh, M.D.  
Michael Belkin, M.D.

CONTRACTING ORGANIZATION: Tel-Aviv University  
Ramat-Aviv  
Tel-Aviv, Israel 69978

REPORT DATE: July 1991

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;  
distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
1a. REPORT SECURITY CLASSIFICATION <b>Unclassified</b>			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT <b>Approved for public release; distribution unlimited</b>		
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE					
4. PERFORMING ORGANIZATION REPORT NUMBER(S)			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION <b>Tel-Aviv University</b>		6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION		
6c. ADDRESS (City, State, and ZIP Code) <b>Ramat-Aviv Tel-Aviv, Israel 69978</b>			7b. ADDRESS (City, State, and ZIP Code)		
8a. NAME OF FUNDING/SPONSORING ORGANIZATION <b>U.S. Army Medical Research &amp; Development Command</b>		8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER <b>Grant No. DAMD17-87-G-7039</b>		
8c. ADDRESS (City, State, and ZIP Code) <b>Fort Detrick Frederick, Maryland 21702-5012</b>			10. SOURCE OF FUNDING NUMBERS		
PROGRAM ELEMENT NO. <b>62787A</b>		PROJECT NO. <b>3M1 62787A878</b>	TASK NO. <b>BA</b>	WORK UNIT ACCESSION NO. <b>208</b>	
11. TITLE (Include Security Classification) <b>TREATMENT OF LASER INDUCED RETINAL LESIONS</b> <b>Subtitle: Laser Induced Retinal Injuries</b>					
12. PERSONAL AUTHOR(S) <b>Nava Naveh, M.D.; Michael Belkin, M.D.</b>					
13a. TYPE OF REPORT <b>Final Report</b>		13b. TIME COVERED <b>FROM 9/30/87 TO 9/29/90</b>	14. DATE OF REPORT (Year, Month, Day) <b>July 1991</b>		15. PAGE COUNT <b>28</b>
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	<b>Vitreous protein levels; Neodymium:YAG laser; Leukotrienes B<sub>4</sub> Lab Animals; Retinal Cornea Burn; Rabbits; RAIII</b>		
<b>09</b>	<b>03</b>				
<b>06</b>	<b>04</b>				
19. ABSTRACT (Continue on reverse if necessary and identify by block number)					
<p>In our previous study we have shown that the ocular response to laser induced ocular injury is characterized by an inflammatory reaction with enhancement in prostaglandin (PG) production and a cellular infiltration. Steroid treatment curtailed the PGs response but did not effect the inflammatory infiltrate. In the present study, we investigated the involvement of leukotrienes B<sub>4</sub> (LTB<sub>4</sub>) in laser induced retinal injury.</p> <p>The following parameters were studied; LTB<sub>4</sub> production by the retina-</p>					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION <b>Unclassified</b>		
22a. NAME OF RESPONSIBLE INDIVIDUAL <b>Mrs. Virginia Miller</b>			22b. TELEPHONE (Include Area Code) <b>301-663-7325</b>		22c. OFFICE SYMBOL <b>SGRD-RMI-S</b>



## FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).